 CSB 327H1, Extracellular Dynamics and Associated Pathologies
Term Test 1, October 10, 2012, 2:10-3:00 P.M, Room 100, UofT Examination Facility

Student Name:_____

Student Number:_____

Total of 30 points worth 30% of the final mark

Multiple choice questions are worth one point each. Enter your answers on the scantron.

1) Which of the following collagen post-translational modifications/events *do not* occur in the ER?

1) The transformation of procollagen trimers into tropocollagens.
2) Cross-linking of the N-terminal domains of type IV collagen with transglutaminase.
3) Cis-trans isomerization of proline residues.
4) Quality control for misfolded collagen molecules.
5) Binding of HSP47 to procollagen molecules.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

2) With respect to kidney filtration, which of the following statements are *correct*?

1) Charge selection is mediated by collagen IV within the GBM.
2) The glomerular Bowman's capsule is fenestrated.
3) Filtration slits are synthesized by podocytes.
4) The GBM of patients with Alport syndrome is composed of an $\alpha1(IV)_2\alpha2(IV)$ network.
5) A developmental shift from an $\alpha3(IV)\alpha4(IV)\alpha5(IV)$ network to an $\alpha1(IV)_2\alpha2(IV)$ network in the GBM occurs after birth.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

3) With respect to the supramolecular composition of collagen fibrils in connective tissues, which of the following heterofibril combinations are *not* formed *in vivo*?

1) $\alpha1(I)_2\alpha2(I)$ and $\alpha1(V)_2\alpha2(V)$
2) $\alpha1(IV)_2\alpha2(IV)$ and $\alpha1(IV)\alpha3(IV)\alpha4(IV)$
3) $\alpha1(II)_3$ and $\alpha1(IX)\alpha2(IX)\alpha3(XI)$
4) $\alpha1(III)_3$ and $\alpha1(I)_2\alpha2(I)$
5) $\alpha1(II)_2\alpha2(II)$ and $\alpha1(IX)\alpha2(IX)\alpha3(IX)$

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

4) With respect to the mechanism of targeting secretory proteins to the endoplasmic reticulum membrane, which of the following statements are *false*?

1) Binding of SRP to the signal sequence halts translation.
2) Signal peptide emerges from ribosomes after binding to the SRP.
3) Positively-charged amino acids are not found in signal peptides.
4) The P54 subunit of SRP contains methionine residues.
5) The signal peptide is a transient extension of the N-terminus secretory proteins.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

5) With respect to the translocation of single-pass secretory proteins across the ER membrane, which of the following statements are *false*?

1) Signal peptides trigger the initiation of mRNA translation in the cytosol.
2) ATP hydrolysis induces a conformational change that promotes the release of SRP.
3) The SRP is released after the ribosome has bound to the translocator.
4) Signal peptides serve as a start-transfer signal.
5) Signal peptide cleavage occurs on the luminal side of the ER membrane.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

6) With respect to type IX collagens, the following statements are *correct*?

1) Type IX promotes the nucleation of type II collagen fibrils.
2) Mutations in type IX collagen are associated with junctional epidermolysis bullosa.
3) Type IX collagen is concentrated in the core of type II collagen fibrils.
4) Type IX collagen cannot be dissociated from type II collagen fibrils with salts.
5) Type IX functions predominantly as a proteoglycan in the vitreous humor of the eye

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

7) With respect to collagen IV assembly, which of the following statements are *false*?

1) C-terminal dimers are stabilized by hydrophobic and H-bonds.
2) N-terminal tetramers promote the binding of type VII collagen to α6β4 integrins.
3) N-terminal tetramers promote lateral association of collagen IV molecules.
4) Chain selection and assembly is directed by amino acid sequences in the C-terminal domain.
5) Lateral association of collagen molecules is promoted by the presence of non-collagenous domains.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

8) With respect to the synthesis and polymerization of type I collagen, which of the following statements are *correct*?

1) Removal of the propeptides decreases the solubility of the collagen molecules.
2) HSP47 is a collagen-specific chaperone.
3) Removal of N-propeptides is obligatory for the initiation of fibril formation.
4) Hydroxylation of proline residues is the rate-limiting step in triple helix assembly.
5) Crosslinking of HO-lysine residues is restricted to the C-terminal propeptide domain.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

9) With respect to epithelial tissue explants, which of the following statements are *false*?

1) When a native lens epithelium with an intact basement membrane is placed apical side up on a collagen gel, the epithelial cells begin to migrate and enter the collagen gel.
2) Basal plasma membrane blebbing is promoted when a corneal explant lacking a basement membrane in place on a Millipore filter.
3) Epithelial polarity is maintained when a corneal explant lacking a basement membrane in place on a Millipore filter containing type I collagen.
4) Embedding a corneal explant in a type I collagen gel induces the expression of type I collagen by the cells undergoing EMT.
5) EMT usually occurs from the apical end of epithelial cells.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

10) Which of the following statements are *false*?

1) Mutations in genes coding for type XVII collagen can lead to nonlethal DEB.
2) The ectodomain of type XVII collagen promotes hemidesmosome assembly.
3) The formation of collagen XVII trimers is initiated by the formation of C-terminal coiled-coils
4) Collagen XVII is often upregulated by migrating keratinocytes.
5) The interaction of collagen XVII with $\alpha 6\beta 4$ integrin promotes cell-matrix adhesion.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

11) With respect to the mouse embryoid body formation, which of the following statements are *true*?

1) The distribution of laminin can be visualized by staining with the fluorescent dye DAPI.
2) Polarization of the primitive ectoderm is independent of basal lamina assembly.
3) Mutations in type IV collagen inhibit stem cell proliferation.
4) The primitive ectoderm gives rise to an amniotic-like cavity.
5) Stem cell differentiation is inhibited by the presence of LIF.

A) 1&2 B) 2&3 C) 3&4 D) 4&5 E) 1&5

Short Answer Questions *(Marks will be deducted for information unrelated to the question you are answering) NOTE: The TAs did give marks for other answers if they were reasonable alternatives or partially correct.*

12) What is the three dimensional organization of type I collagen fibers in the skin? Why? (1 pt)

Organization: Weave-like

Why? To provide tensile strength in all directions that forces are applied

13) With respect to collagen fibrillogenesis, what is the functional similarity between type V and type XI collagens? (1 pt)

They act as nucleators to promote the initiation of collagen fibrillogenesis

14) Why is the cartilage normal in organisms with dermatosparaxis disease? (1 pt)

ADAMTS-2 in not expressed in cartilage ADAMTS-3 is expressed in cartilage

15) Which do you predict would have a higher content of proline residues in their fibrillar collagens, cold-water fish or warm-water fish? Why? (1 pt)

Fish type? Warm-water fish

Why? A higher content of proline residues will result in a higher HO-proline content, which will stabilize the triple helices at higher temperatures

16) What is a possible consequence on embryonic development of inhibiting the glycosylation of type I collagen lysine residues? (1 pt)

A decreased rate of collagen internalization and/or interaction with other ECM components

17) What cellular process promotes the transformation of the first extracellular compartment into a secondary compartment? (1pt)

Retraction of plasma membranes to promote bundle formation

18) Why is rat-tail collagen digested with proteases before fractionation by SDS-PAGE? Why is the β band visible as a doublet by Western blot? (2 pts)

Why protease digestion? The fibrils are too large to solubilize and/or penetrate into the gel

Why a doublet? It appeared as a doublet because the fibrils were not completely digested. Consequently two bands were visible – one representing a dimer composed of α1α1 chains, the other composed of α2α1 chains.

19) Which of the following would cause a more severe osteogenesis imperfecta phenotype; a Gly400Val or a Gly800Val mutation in the pro-α1(I) collagen chain? Why? (2 pts)

Which? The Gly800Val mutation
Why? Triple helix formation begins from the C-terminal end. Interference with folding will result in increased and aberrant hydroxylation and glycosylation resulting in defective helices.

20) Provide two reasons why the visceral endoderm appears disorganized in the absence of HSP47? (2 pts)

1: Decreased CIV secretion (50% decrease)
2: Increase sensitivity to proteases

21) What method would you use to differentiate between type II and type IX collagen molecules in cartilage? Why? (1 pt)

Method: TEM using antibodies against the collagens tagged with different size gold particles

Why? The different size gold particles are visible under TEM, enabling you to differentiate between the different collagens.

22) Which type IV collagen mutation(s) result in an equal probability of Alport syndrome between males and females? Why? (1 pt)

Which? Mutations in the α3 and α4 chains of CIV
Why? The genes are found on autosomes

23) Name two intracellular mechanisms that contribute the elimination of mutated α-chain from bone forming cells? (1 pt)

#1. ERAD (proteasome degradation)

#2. Autophagy

24) In normal tissues, where are pN-collagens associated with collagen fibrils? What have researchers concluded regarding their location? (2 pts)

Where? Outer surface of the fibrils

Conclusion: That cleavage of N-propeptides is regulated to limit the thickness of collagen fibrils

25) When embryonic heart explants were placed on a collagen gel, dense tracks of collagens were observed after 4 days. Which of the following were used to make the collagen: "procollagen" or "tropocollagen"? Why? (1.5 pts)

Which? Tropocollagen

Why? Procollagen molecules cannot form polymers in vitro. Hence dense tracks would not be visible if procollagen was used.

What intracellular event promotes the pulling force? (0.5 pt)

Actinomyosin contractions